Final progress report on research proposal 2015-1662 (First year of the project)

1. **Summary**:

Project Title: Determine the impact of cluster thinning and cluster zone leaf removal on the hormone content of Pinot noir grape berry.
Principal investigator: Laurent Deluc

During the past six months we have succeeded to reproduce the flower-to-berry monitoring procedure developed in our lab with similar outcomes. The justification of this procedure is to mitigate the extreme variability of flowering events in a cluster that is assumed to explain the berry variability. Using this procedure, we were able to distinguish "early" berries (emerging from early flowering events) from "late" berries (emerging from late flowering events). Previous observations in our group suggested that flowering time was not the major contributing factor of the ripeness variability at mid- *véraison* stage (50% of berries are green and 50% are colored) . This was confirmed again this year via the monitoring of several phenological parameters on "early" and "late" berries. We also confirmed that the seed weight relative to seed weight (SB) better explained the ripeness of individual berries at mid-*véraison* stage regardless of whether berries were categorized in the early or late berry groups. Interestingly, by monitoring berry size and berry weight, we also found that "early" and "late" berries rapidly overlapped their growing curves during the early stages of the growing season (week 3 to week 6 after bloom), which suggests a developmental mechanism to mitigate developmental variability among berries of a cluster. On the other hand, ripeness variability at *véraison* was not associated with berries being "early" or "late" as both berry groups had a wide range of ripeness level at mid-véraison stage (sugar and pigment content). We also validated the effects of two viticulture practices (cluster thinned and fruit-zone leaf removal) on sugar and pigment contents regardless of whether berries were "early" or "late". In vines with clusters thinned at 0.5/shoot, both accumulation of sugar and pigment contents were significantly higher in berries during the late stages of the ripening. For the fruit-zone leaf removed, only pigment content was significantly increased in sun-exposed clusters during weeks 12-15. The fine screening we performed to mitigate the developmental variability of berries has been successfully conducted and we are in the second phase of the project this year, which is the quantification of hormone and metabolite in control, cluster thinned, and fruit-zone leaf removed grapevines. The first series of hormone quantification indicates that the accumulation of ABA and GA1 during the pre-ripening stages and at the ripening onset tends to be more associated with the SB ratio than the flowering time. This may suggests a minor role of flowering in determining the dynamics of certain hormones if not all. A series of second on-going hormone analysis will clarify this assumption.

2. **1st annual Report (June 2014-January 2015):**
3. **Project Title**: Determine the impact of cluster thinning and cluster zone leaf removal on the hormone content of Pinot noir grape berry - Proposal number: 2015-1662

Project's leader: Dr. Laurent Deluc, Department of Horticulture, Oregon State University - delucl@hort.oregonstate.edu - 541-737-4356

4. **Principal Investigator/ Cooperators**:

Co-Principal Investigator: Dr. Elizabeth Tomasino, OSU, Department of Food Science and Technology at Oregon State University. elizabeth.tomasino@oregonstate.edu - 541-737-4866
Cooperator: Scott Robbins, Farm manager at Oregon State University - 541-737-5483

5. Objectives(s) and Experiments Conducted to Meet Stated Objective(s):

1) Determine the hormone dynamics of grape berry from flowering to harvest.

2) Estimate the effects of cluster thinning and leaf removal on the hormone profiles during the ripening phase.

The primary objective of the research project is to provide a clear snapshot of the hormone dynamics in a grape cluster under different vineyard managements. Because the hormone content in grape berry is often specific to a developmental stage the biological noise caused by natural uneven grape berry development may obscure the expected outcomes. This makes difficult to draw any definite conclusions. To prevent this major hurdle, we developed a **flower-to-berry tracking system** enabling the monitoring of individual berries that emerge from similar flowering events (Early, mid-time, and late flowering). This will guarantee a better assessment of the hormone content, their dynamics during the growing season from berries that originate from similar flowering events.

6. Summary of Major Research Accomplishments and Results by Objective.

For the past six months, the major research accomplishments have been focused on three aspects of the proposed research: **1)** Selection of individual berries used further for the hormone quantification, **2)** Measurement of ethylene during the pre-ripening stages (weeks 6 to 7), and **3)** Effect of two viticulture practices (Cluster Thinning and Leaf removal) on the phenology of grape berries. All these accomplishments are relevant to the two objectives defined above. For this reason, the structure of this section mostly describes the generation of the phenological data that will be used for the hormone and metabolite, which is scheduled to be performed during the next months. Another aspect that needs to be mentioned is the procedure of the flower-to-berry monitoring, which has involved the collections of two berry populations originating from Early (Day 1 and 2) and Late (Day 8 to 10) flowering events. The justification of these two collection dates stems from new findings in our lab that suggest a possible readjustment in the developmental curve of berries originating from late flowering events enabling them to "catch up" with those that had flowered early within the cluster (Vondras et al., 2015).

6.1. Flower and berry phenology.

6.1.a Experimental design and tagging procedure: In June 2014, we conducted the experiment in the block B of 2006 Pinot noir at the Woodhall experimental OSU. As proposed by the panel, we set up the experiment in a randomized complete block design with three blocks and each treatment (cluster thinning, leaf removal, and control) within a block (**Figure 1**). In the late stages of inflorescence development, prior to anthesis, inflorescences at similar developmental stages were monitored until their first day of anthesis. Two plants per each testament replicate were flagged. Within each plant, two inflorescences selected on each side of the canopy were identified. Once the first of anthesis recorded, the procedure of tagging begun on a daily basis by tagging individual flowers with color-coded strings for each inflorescence (**Figure 2**).

6.1.b Phenology of anthesis in 2014: Among the 36 inflorescences identified, seven were not proceeded until the end of the experiment due to unexpected events in the field (flowers that never opened, or the shoots bearing the inflorescences broke). Among the 29 remaining inflorescences, 75% of the inflorescences had their first day of anthesis June 11th while the remaining 25% had their first open flower events two days later. The majority of inflorescences has had an average of ten days of consecutive flowerings per inflorescence. Few of them had shown a " pause " of two days shifting slightly later their development by three days. An average of ~190 flowers per cluster were tagged. One tagging event was estimated to be missed per day for each inflorescence. The homogeneity of flowering events per cluster (**Figure 2**) was assessed by dividing flowering days into three main categories of flowering (Early [days 1-2]; Mid-time [days 3-7]; and Late [days 8-10]. We did not observe any relationship between flowering events (Early, Mid-time, Late) and the position of the flower within the inflorescence (basal, mid-position, and distal). In general, early and late flowering events contributed to 5-70% and to 15-95% of the total flowering events per inflorescence, respectively. Mid-time flowering days contributed at most to 45% of the total flowering events per inflorescence. This indicates that the majority of the flowers open in this block either during the early or late stages of anthesis. The record of the average temperature in the morning did not show any correlation between the temperature of the day prior to- and at the flowering event and the percentage of open flowers during that day (Data not shown).

6.1.c Collection and phenology of the berries from flowering until harvest: Fifteen weeks separated the first day of flowering from the collection of last berries at mature stage. In order to maintain a reasonable source-sink relationship between berries of a cluster, we collected one individual berry per cluster (n=2)/per plant (n=2)/flowering events (early, mid-time, late)/block/ treatment at week 2, 4, 5, 6, 7, 8, 9, 10, 12, and 15 for a total of ~580 berries collected in separate bags and stored in dry ice prior to storage at -80°C until use. These berries were collected each week during the same period of the day (from 2:00 to 3:00PM). From each individual berry, a series of phenological parameters was measured to monitor the developmental stage of each individual berry at each collection date. This includes growth and ripening parameters that are listed in **Table 1.** Because berries from week 2 were too small, we could not measure the parameters except for berry size and berry weight.

6.1.d Selection of the best-fit berries to a developmental stage: To select the best individual berries for the hormone quantification, we performed a linear discriminant analysis (common covariance between predictors) on the different phenological parameters listed in Table 1. Both populations of berries (early or late), collected per treatment replicate, were analyzed to fulfill the three assumptions. The ability of discriminant analysis is to extract discriminant functions capable of producing accurate classifications. This is enhanced when the assumptions of normality, linearity of the distribution (Multicollinearity), and the homogeneity of the variance are satisfied (General Linear Model Method in JMP package - SAS program). In this context, outliers individual berries and phenological parameters that do not fulfill these assumptions were discarded for the selection of best candidate berries (Table 2). Because the fruit development is not a linear process, some predictors were more relevant during the berry growth while others were found more associated with ripening. We tested the relevance of each by defining four developmental phases that are 1) active berry growth (weeks 4 to 6), 2) transition to lag phase and lag phase (weeks 7 and 8), 3) ripening transition (weeks 9-10) around *véraison*, and 4) late phases of ripening until harvest (weeks 12 to 15).

6.1.e Phenology of the berries from early and late flowering events in control plants: Once the best candidates classified via the discriminant analyses, we plotted several phenological parameters to determine if their dynamics vary between « early » and « late » berry populations during the growing season (**Figure 5**). In most cases, the degree of variability among the berries was relatively low across the season. However, a relatively greater variability was found when we measured ripening parameters such as sugar and pigment content before and during *véraison* (weeks 8-10). This ripeness variability was found to be not correlated to flowering events the berries originated from, which indicates little influence of the flowering time to explain the ripeness variability among berries of a grape cluster at *véraison* stage (Vondras et al. (2015) submitted to PlosOne). Overall, the discriminant analyses identified berries with similar developmental state, which was the main objective of this multivariate analysis. Interestingly, while a minimum of six days separated early from late flowering events, the overall dynamics of most of the parameters appeared to overlap over the grape berry development. Only the growth of the pericarp (berry size and berry weight) seemed to be significantly different during the early stages of berry growth, from week 4 to week 6, but the dynamics of both berry populations tend to converge thereafter (**Figure 5**). This might be an indication for a readjustment in the developmental curves of both the early and late berries during the early stages of berry growth, which could involve possible some hormone readjustment between the berry populations. It will be interesting to see if this « merging » is reproducible from year to year and if a change in hormone content is associated with this merging. The ripeness variability observed during *véraison* was found to be associated with the seed weight-to-berry weight ratio. This confirms a recently accepted paper by our group that indicates a strong relationship between the S:B ratio and the ripening states of berries within the *véraison* stages, which can be used to identify berry individuals that follow a similar ripening progression (Gouthu and Deluc, 2015). Among the individual berries collected during the weeks 7-8-9 and regardless of the flowering events they originate from, berries with higher sugar and pigment contents were the one with the lowest S:B

ratio; conversely the berries that were lagging has the highest S:B ratio (**Figure 6**). This relationship was found to be associated with changes in the auxin pericarp content of berries Gouthu and Deluc, 2015).

6.1.f Impact of the treatments on the berry growth and ripening of berries emerging from early and late flowering events: The effects of two viticulture practices (cluster thinned and fruit zone leaf removal) were evaluated on the same parameters for growth and ripening in the two berry populations during the growing season (**Figure 7**). In vines with clusters thinned at 0.5/shoot, both accumulation of sugar and pigment content was significantly higher in early and late berry populations during the late stages of the ripening (week 12 and 15). For the leaf removal treatment, only pigment content was significantly increased in sun-exposed clusters during the weeks 12-15.

6.2. Environmental factors assessment.

Leaf water potential on the shoots bearing the flagged clusters was measured at each week we collected the berries according to standard procedures (**Figure 8A**). The leaf water potential did not go below -0.9MPa across the season regardless of the treatment. The block B of the vineyard is known to have a deep soil with high water hold capacity from winter and spring rains, which limits the water regime to its minimum during the entire growing season mostly to no irrigation during the growing season. No heat wave was recorded during the growing season as well (**Figure 8B**). The tentative attempt to record the temperature of the different individual berries was not successful. Technicality issues with the instrumentation (thermocouple probes) and berry necrosis events caused by the wounding following the insertion of the probes into the berries have prevented the generation of reliable data. The next two years, we will use a Mini InfraRed Thermometer gun with laser pointer to measure the temperature of the individual berries collected during the season (Spectrum Technologies, Inc. IL, USA). The advantage of the instrumentation is to provide a non-destructive and accurate measures of the internal temperature of berries. Post-season assays using semi-thawed individual berries were conducted and the validated the high reproducibility of the measures a long a population of 200 berries randomly picked (data not shown). We will complement the measure of the internal berry temperature by measuring the skin surface temperature using a digital traceable thermometer (VWR, USA). The assessment of the light interception using the ceptometer was not resolutive enough to provide reliable measures at the cluster level. Another procedure that remains to be defined at that stage will be investigated this year.

6.3. Ethylene quantification.

For some limitations in terms of tissue material, the ethylene quantitation was performed in berry emerging from mid-time flowering events. Ethylene quantitation using GC-Flame Ionization Detector (FID) was attempted on grape berry samples from 2014 following a protocol described in Sun et al. (2010). In short samples were enclosed in air tight glass containers with a PTFE septum and frozen until analysis. Samples were brought to room temperature and left at 20°C for 2 hours. One mL of headspace was injected into the GC-FID (model Shimazdu, GC-2014, USA). No ethylene was measured in any of the grape berry samples following this method. With few

remaining samples an additional methodology for measurement of ethylene in grape berries was attempted in which samples were sealed in the airtight glass containers with PTFE lids and held at 100°C for 90 minutes (Hilt and Bessis, 2003). One mL of headspace was then injected into the GC-FID. Ethylene was not detected using this methodology either and the grape berries chosen were those harvested towards the end of the season, which suggests they should have higher levels of ethylene (Sun et al., 2014). This suggests that the levels of ethylene produced in Pinot noir grapes was so low that they could not be detected with the GC-FID. Either additional method development is required, in which larger volumes of berries are measured or a different sampling method is used, or the amount of ethylene produced from these grape berries at these times is negligible.

6.4. Preliminary data of the hormone analysis in control plants.

From Figures 5, 6, and 8, we observed that the dynamics of several phenological parameters during the grape berry development overlap regardless of the flowering time (early and late flowering events), which might indicate a minor role of flowering in determine the developmental stage of individual berries. Seed is known to affect growth during the first phase of berry development and seed number was found to correlate berry size and berry weight (Ristic and Iland, 2005). Differences in seed weight to berry weight ratio of berries (SB) before the ripening onset were recently shown to result in differences in ABA and auxin accumulation in the pericarp tissues (Gouthu and Deluc, 2015). Altogether, these different results might suggest a preeminent role of seed in determining the rate of growth and the timing of ripening onset as it is proposed in Gouthu and Deluc (2015). To evaluate which between flowering time and seed content relative to berry weight is responsible for the developmental stage of a berry, we included the SB ratio as additional parameter. We measured the Seed Weight to Berry Weight (SB) from « Early » and « Late » berry groups berries in control plants at week 6,7,8, and 9 and we only selected berries with Low S:B (First quartile of the overall distribution) and those with High S:B berries (last quartile of the overall distribution). Overall, we observed that berries form « Early and Late » flowering events had similar ranges of Low and High S:B ratios at each week analyzed, respectively (**Figure 9A**). The overall declining trend of the S:B ratio towards the ripening onset, caused by the conclusion of the seed growth and/or the second growth of the pericarp at the ripening onset, was also observed (**Figure 9A**).

6.5. Hormone analysis including Early and Late and Low and High SB ratios during the pre-ripening stages and at the ripening onset.

To simplify the content of this section, only the most relevant and finalized result will be discussed. The bioactive forms of ABA, auxin, and gibberellins (GA1,3,4, and 7) were measured during the pre-ripening stages (Week 6, 7, 8, and 9) in the pulp tissues of control plants according to their flowering events (Early and Late) and their seed weight relative to berry weight (High and low). The choice of these three hormones are based upon their impact on different aspects of growth and ripening. While Auxin and Gibberellins are known to promote berry growth and to delay ripening ABA was found to promote ripening in grape berry. It also assumed that seed is a reservoir of Auxin that could be transported to the surrounding tissues. As recently validated by Gouthu and Deluc (2015), pericarp tissues of berries with higher SB contain greater levels of auxin and lower levels of ABA; conversely pericarp tissues of berries with lower SB ratio contain less auxin and more ABA per g of berry. We therefore decided to

focus on these three hormones to better evaluate the contribution of seed and flowering times to the hormonal content of berries.

With respect to ABA and gibberellins' compounds, the quantification of these two hormones confirmed our expected outcomes. For ABA, at week 9 (ripening onset in our data), we observed a greater accumulation of ABA in berries with lower SB ratio compared to those with a higher SB ratio *regardless of their flowering times* (**Figure 9B**). For GAs, only GA1 and GA4 were quantified. GA3 and GA7 were to low to provide accurate measures. While GA4 did not show difference between « Early and Late » berries and low and high SB berries (Data not shown), GA1 was higher at week 8 in berries with high SB ratio compared to low SB berries (**Figure 9C**). In fruits, Auxin was found to enhance GA1 biosynthesis probably by decreasing the GA inactivation, thereby leading to higher level levels of GA1. Therefore, the increase in GA1 observed could be associated to a greater level of auxin in these berries. Due to interfering compounds in the matrix of the pulp tissues, quantification of Auxin did not yield accurate measures. We are currently troubleshooting the analytical method to solve the issue. An adjustment in our protocol is currently tested to retrieve Auxin from our samples.

6.6. Overall Research Accomplishments.

During the past six months, we made significant progress and we successfully achieved our major goal for the first year, we developed a fine-tuned methodology to circumvent biological variability, which is essential in our final goal to accurately measure several classes of hormones from individual berries of a grape cluster. We also confirmed that the influence of flowering time within a grape cluster may not be as intuitive as we believe in terms of defining a developmental stage. We found that lagging berries, caused by late flowering, appear to adjust their developmental curve early in the season during the berry growth (around weeks 4-6), probably through differential hormone accumulation. The quantification of hormones will clarify this assumption. On the other hand, this readjustment does not appear to influence the inherent ripening variability observed at *véraison*, which appears to be more associated with the weight of seed(s) relative to the berry weight (SB) and the first series of hormone analysis tends to support this assumption. We also validated the effects of the two practices (cluster thinning and fruition leaf removal) that are part of the experimental design and we look forward to seeing their impact on the hormone dynamics. The hormone analysis including the detection of other hormones (JA, SA, and BRs) and the effect of cluster thinning and leaf removal is currently on going and is scheduled to be run the next two months.

7. Outside presentation of research. One poster was presented at the last Grape Day meeting in Corvallis. In the summer of year 3, we will develop an event at the Woodhall Experimental station to discuss about the research concept (flower tagging, hormone dynamics) with the Industry. One abstract for poster presentation will be submitted to the ASEV this year and to the Grape Day at OSU.

8. Research Success Statements.

The PI's research program focuses on grape berry genomics. One of the major scopes of the research group is to understand the role of plant hormones in the control of grape berry

development and how environmental factors or viticulture practices may affect the dynamics of plant growth regulators. However, the tremendous developmental variability existing in a grape cluster has hampered research progress in this scientific area. The outcomes of this research project will provide the grower industry with a clearer time and tissue specific snapshot of the accumulation of plant growth regulators during grape berry development. This could be a very useful information to design targeted practices during the growing season involving the use of plant growth regulators with the final objective to manipulate growth and ripening of grape berry. The impact of two viticulture practices will be evaluated on the dynamics of these hormones, which could be useful in order to identify alternative strategies to substitute these cost-expensive practices.

9. Funds status.

Approximately 75% of the budget was spent in salaries for students during the tagging and collection procedure (~$18,000), the hormone analyses including extraction, purchase of internal standard and the use of instrumentation at the Mass Spectrometry Facilities at OSU for a total of $11,000. The travel expenses from OSU to Woodhall, the purchase of liquid nitrogen to store, the separation and the crushing of the tissues were estimated at around $3,000. One student was recently recruited to pursue MS program under my supervision.

Literature cited:

Gouthu S., and LG. Deluc (2015). Timing of ripening initiation in grape berries and its relationship to seed content and pericarp auxin levels (2015). *BMC Plant Biology* (Accepted for publication).

Hilt C., and R. Bessis (2003) Abscission of grapevine fruitlets in relation to ethylene biosynthesis. Vitis 42: 1-3

Sun L., Zhang M., Ren J., Qi J., Zhang Q., and P. Leng (2010). Reciprocity between abscisic acid and ethylene at the onset of berry ripening and after harvest. BMC Plant Biology 10: 257

Vondras AM., Gouthu S., Petersen AR., and LG. Deluc (2015). Time of flowering and seed content contribute to variable entry of Pinot noir fruits into the ripening phase. (submitted to *PlosOne*)

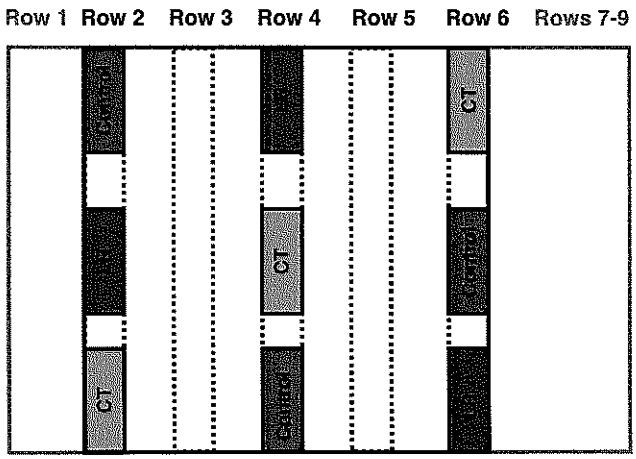

Figure 1: Experimental design in the vineyard block consisting of 52 vines per row (9 rows in total). Control plants consist of eight grapevines per block have shaded clusters with two clusters per shoot. Leaf removed plants (LR) are eight grapevines with exposed clusters with two clusters per shoot. Cluster thinned (CT) are eight grapevines per block with shaded clusters and 0.5 cluster per shoot. Remark: Two plants (1 and 2) selected within the pool of eight vines per block were flagged and two inflorescences per plant on the east and west side of the canopy were used to tag the flowers.

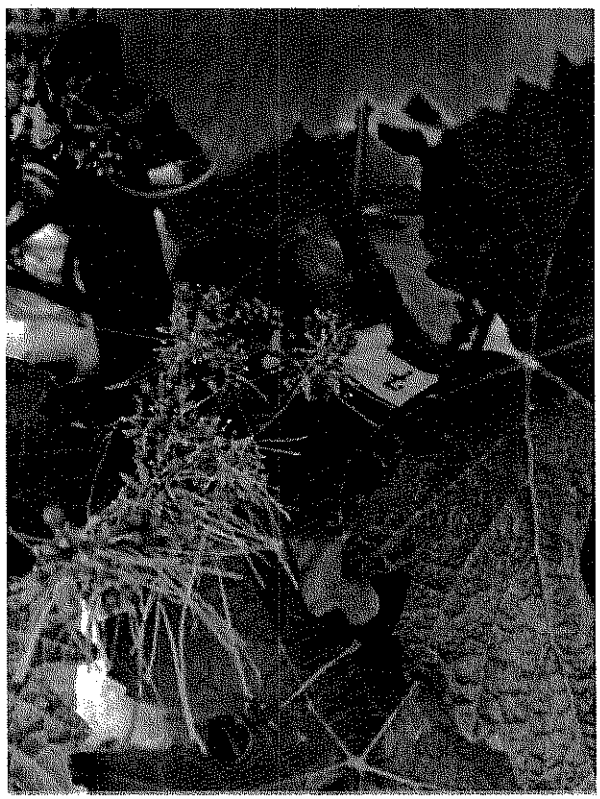

Figure 2: Photography depicting the tagging procedure on individual flowers.

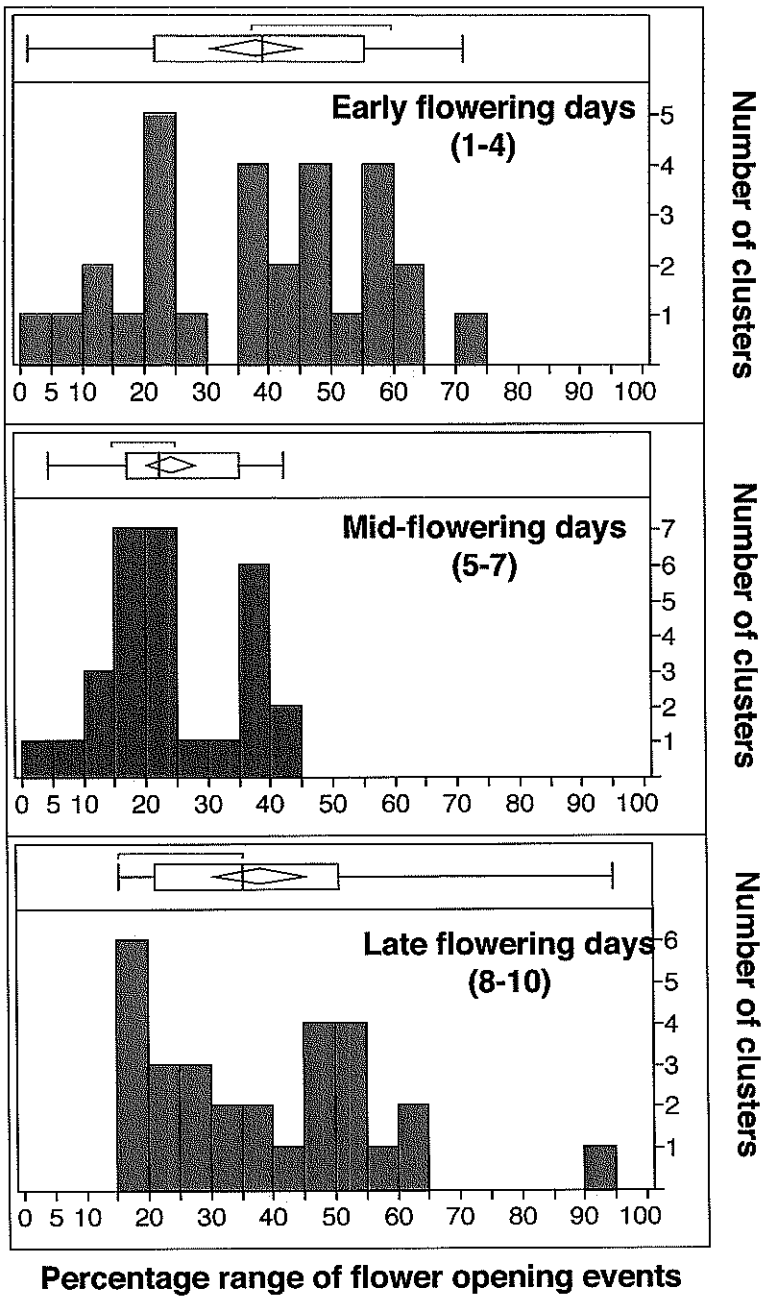

Figure 3: Contribution of early, mid, and late flowering days to the overall flowering events per cluster.

		Week 2	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	Week 15
Color	Lightness	-					X	X	X	X	X	X
	Chroma	-					X	X	X	X	X	X
	Hue	-					X	X	X	X	X	X
	a	-					X	X	X	X	X	X
	b	-					X	X	X	X	X	X
Sugar	Brix				X	X	X	X	X	X	X	X
Berry Growth	Pedicel diameter	-	X	X	X	X	X	X	X	X	X	X
	Berry diameter	-	X	X	X	X	X	X	X	X	X	X
	Berry weight	-	X	X	X	X	X	X	X	X	X	X
Seed Growth	Seed Number	-	X	X	X	X	X	X	X	X	X	X
	Seed Weight	-	X	X	X	X	X	X	X	X	X	X
	Seed Color	-	X	X	X	X	X	X	X	X	X	X
	Seed Shape	-	X	X	X	X	X	X	X	X	X	X
Seed to berry growth	S:B	-	X	X	X	X	X	X	X	X	X	X

Table 1: List of phenological parameters measured to estimate the developmental stage of berries. **X** describes the use of given parameter to a given week. S:B: Seed Weight-to-Berry Weight.

Predictors	Week 4 to 6			Week 7 to 8		
	Normality	Unequal Variance	Multicollinearity	Normality	Unequal Variance	Multicollinearity
Berry Diameter	X	X	Berry Weight	X	X	Berry Weight
Pedicel Diameter	X	X	X	X	X	X
Berry Weight	X	X	Berry Diameter	X	X	Berry Diameter
Sugar content	X	-	X	X	X	X
Seed Number	X	-	Seed Weight	-	-	-
Seed color	X	-	Seed Shape	-	-	-
Seed Weight	-	-	Berry Weight	-	-	-
Seed Shape	-	-	Seed Color	-	-	-
S:B	X	X	X	X	X	X
h	-	-	-	-	-	-
L	-	-	-	-	-	-
C	-	-	-	-	-	-
a	-	-	-	-	-	-
b	-	-	-	-	-	-
Color Index	-	-	-	-	-	-

Predictors	Week 9 to 10			Week 12 to 15		
	Normality	Unequal Variance	Multicollinearity	Normality	Unequal Variance	Multicollinearity
Berry Diameter	X	X	Berry Mass	X	X	Berry Weight
Pedicel Diameter	X	X	X	X	X	X
Berry Weight		X	Berry Diameter	X	X	Berry Diameter
Sugar content	-	X	b, h, color index	X	X	X
Seed Number	-	X	Seed weight	-	X	X
Seed color	-	-	X	-	X	X
Seed Weight	-	X	Seed number	X	X	X
Seed Shape	-	X	X	-	X	X
S:B	X	X	X	-	X	X
h	-	X	L, a	-	X	a
L	-	X	h, color index	X	-	b, color index
C	X	X	X	X	-	color index
a	-	X	h	X	X	h
b	X	X	a, h	X	-	L
Color Index	-	X	L, h, brix	X	-	L, C

Table 2: Example of the assessment for predictors in control vines to meet the three required assumptions for a linear discriminant analysis. **X** indicates that the predictor meets the standard for Normality of the distribution, Unequal variance among the populations, and Multicollinearity. - indicates that the predictor did not meet neither the standard nor was used at that particular range of stages.

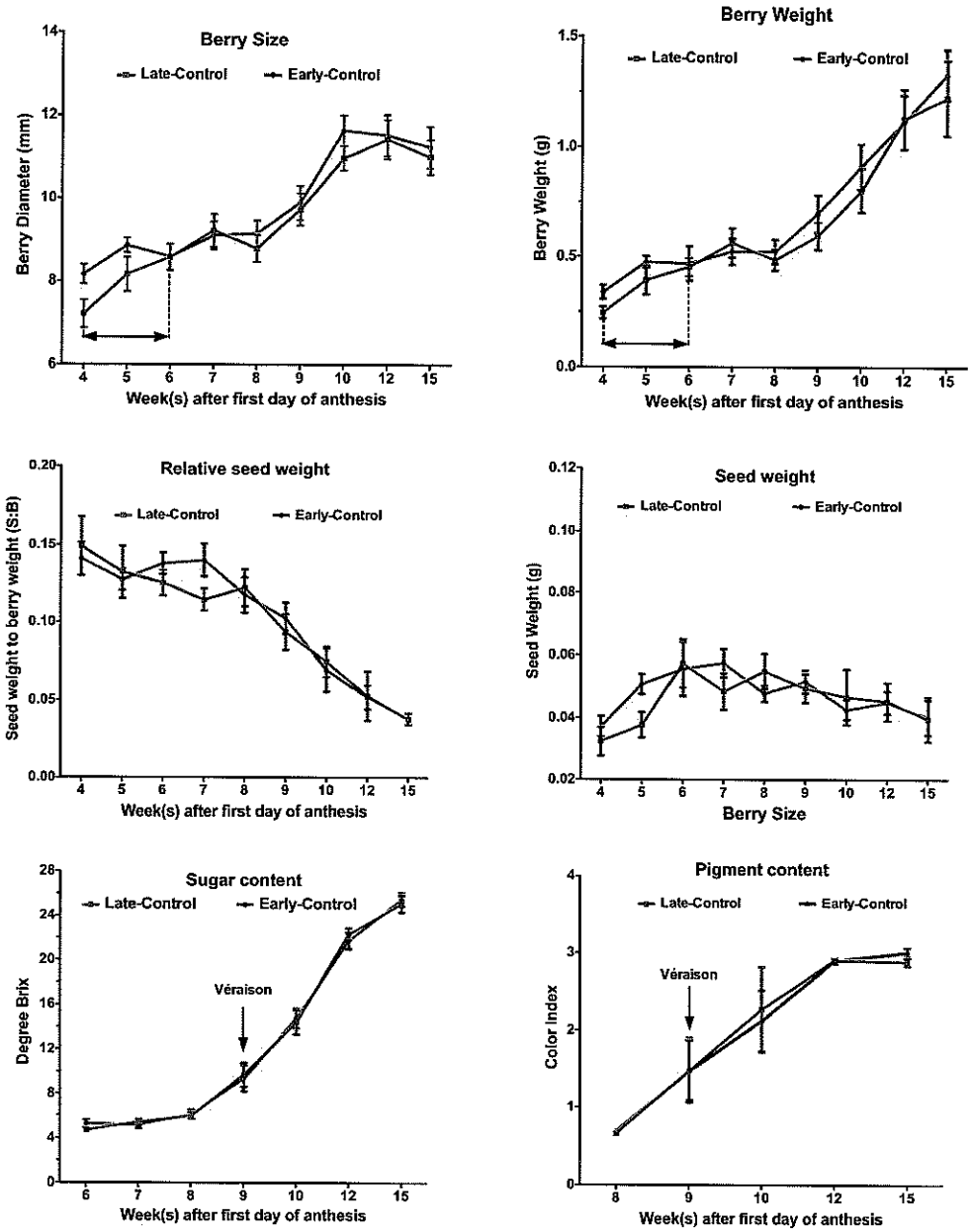

Figure 5: Dynamics of six berry growth and ripening parameters of berries emerging from early (Day 1-2) and late (Day 7-10) flowering events (n=12). Weeks are determined based on the first day of flowering per cluster. **Remark**: i) Double arrows depict the merging between the two berry populations for the berry size diameter, berry weight and S:B parameters, ii) Seed parameters (seed weight and relative seed weight (S:B) measures) are based on one seeded berry populations.

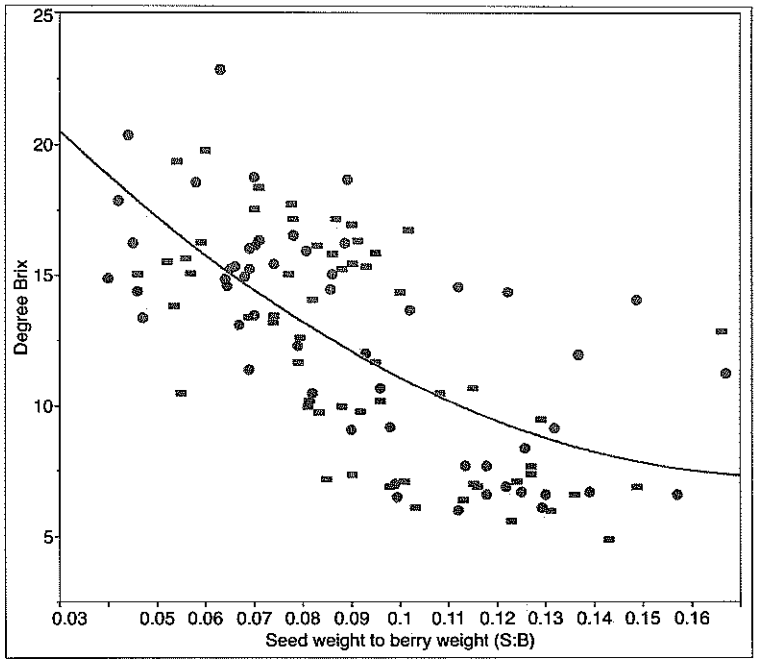

Figure 6: Bivariate analysis between sugar content and S:B ratio during *véraison* stages (week 9 and 10). $R^2 = 0.65$

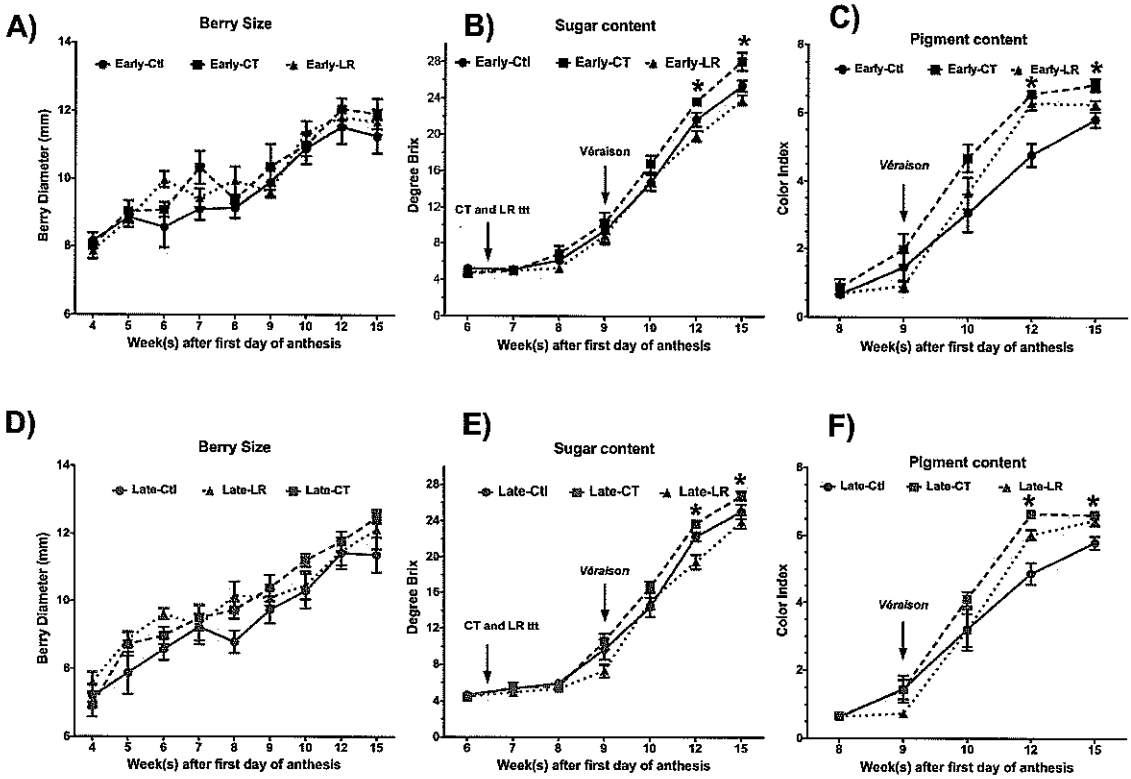

Figure 7: Dynamics of three berry growth and ripening parameters of berries emerging from early (A,B,C) and late (D,E,F) flowering events in control grapevines, and under fruit zone leaf removal and cluster thinned conditions. Weeks are determined based on the first day of flowering per cluster. **Remark**: **CT**: Cluster thinned, **Ctl**: Control plants, **LR**: Leaf removal. The asterisk describes significance differences between at least one of the pairwise comparisons at each developmental stage (Tukey's HSD test).

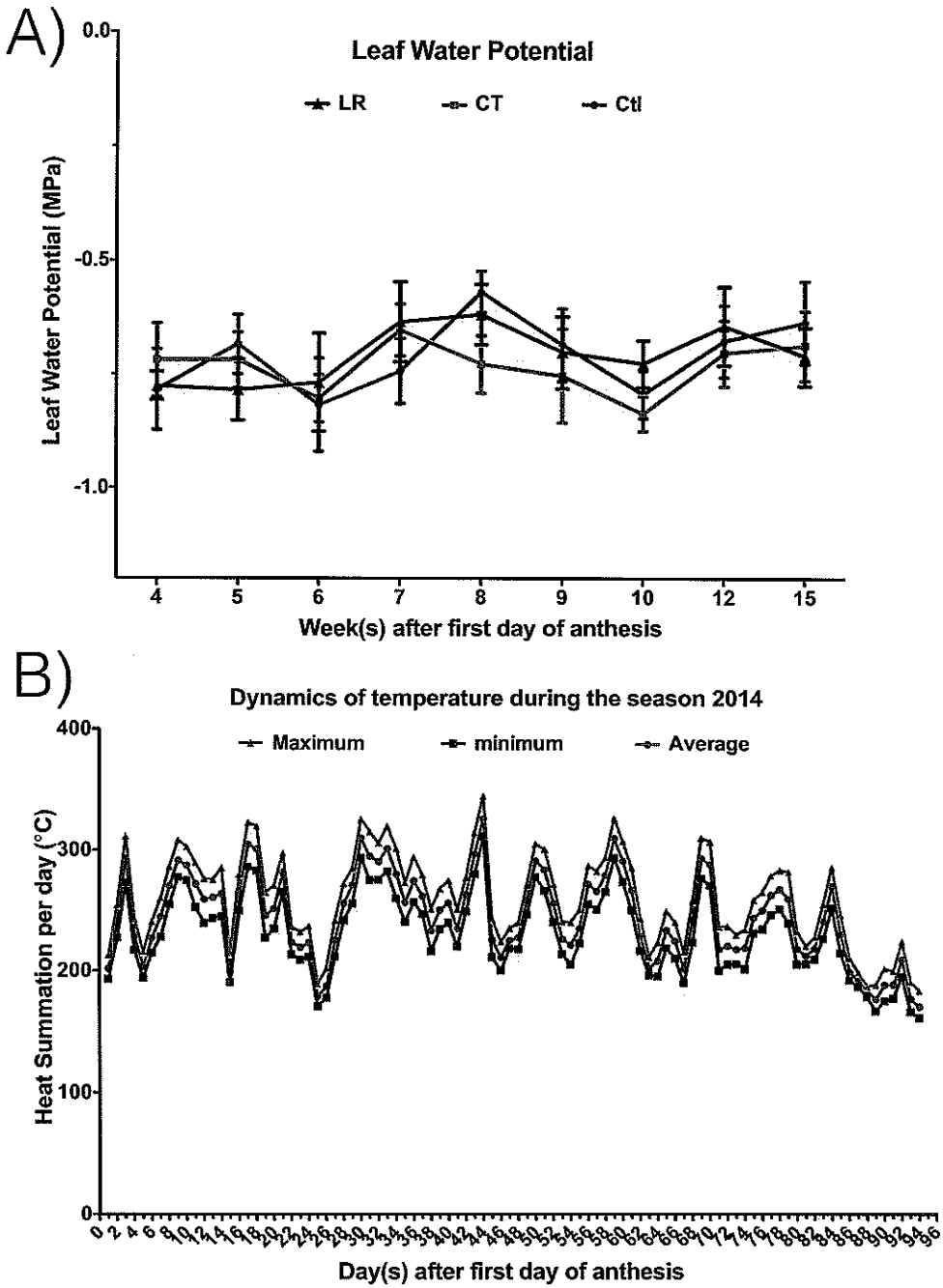

Figure 8: Environmental factors in the block B of the vineyard. **A)** Leaf water potential in control (**Ctl**), cluster thinned (**CT**) and fruit zone leaf removed (**LR**) grape vines. **B)** Heat summation per day from the first days of anthesis until the collection of the mature clusters. Heat summation is the sum on a daily basis of the average, maximum, minimum temperatures recorded every two hours.

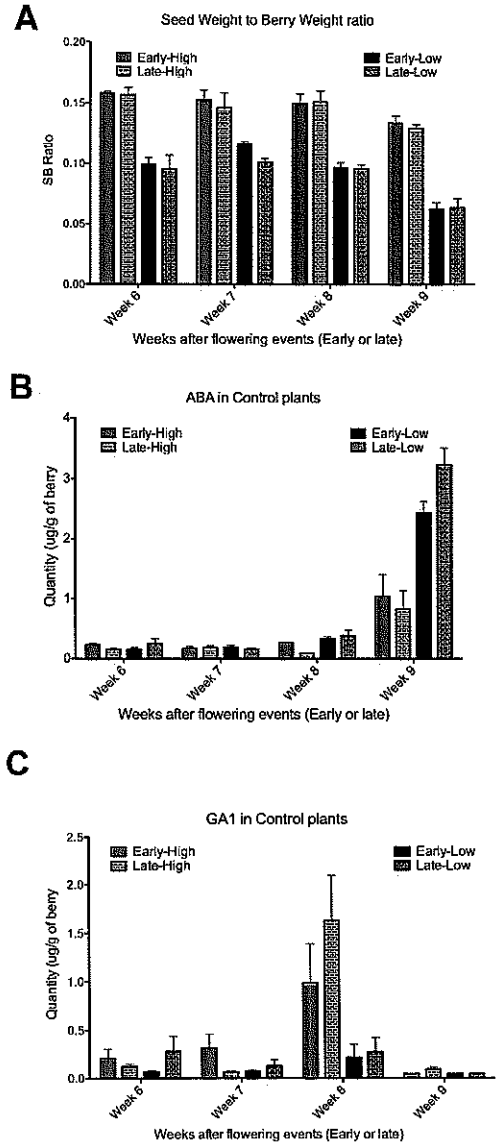

Figure 9: Hormone profiling in berries from Week 6 to Week 9 in control plants (**Ctl**).
A) Seed Weight to Berry Weight ratio in berries from Early and Late flowering events in **Ctl** plants from Week 6 to Week 9. **B**) Dynamics of ABA. **C**) Dynamics of Gibberellin Acid 1. **Remark**: Early-High: Berries emerging from Early Flowering events with higher SB ratio. Late High: Berries emerging from Late Flowering events with Higher SB ratio. Early-Low: Berries emerging from Early flowering events with lower SB ratio. Late-Low: Berries emerging from Late flowering events with lower SB ratio.